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Effect of microbes on food selection by *Lumbricus terrestris*

BY

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INTRODUCTION

In 1881 DARWIN demonstrated that earthworms readily distinguish between different food substances. Since then many attempts have been made to correlate earthworm food variables with palatability. The most detailed investigation to date is that of SATCHELL and LOWE (1966) who were able to show an association between a number of factors and food preference, including content of N, soluble carbohydrates and phenolic substances. However, a complete explanation for the clear choices shown by earthworms for particular food materials is not yet available. Probably all of the factors shown to be correlated with palatability have a role to play in final selection, possibly in different combination depending on environment.

BARLOCHER and KENDRICK (1973 a and b) demonstrated that food preferences of the freshwater crustacean *Gammarus pseudolimnaeus* could be manipulated by an appropriate choice of fungal inoculum on their dead leaf diet. Only WRIGHT (1972) has considered the part played by microbes in influencing earthworm food palatability, although SATCHELL and LOWE (1966) suggested that microbial action was the main process which removed 'distasteful substances' from dead leaves. The following experiments were designed to make a further contribution to our understanding of the relationship between earthworms, their food substrate, and microbes.

I. — MATERIALS AND METHODS

Ten opaque plastic containers, each 30 × 20 × 20 cm deep, were filled to a depth of 5 cm with finely-sifted, sterile garden soil. Into each container were placed 14 spe-

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cimens of *Lumbricus terrestris* (each from 1.5 to 2.0 g fresh weight). The soil was then watered and the containers covered with polythene to reduce evaporation. They were left at room temperature for one week to allow the earthworms to acclimatise.

Three common soil microbes were used: two fungi (*Mucor hiemalis* and a *Penicillium* sp.) and one bacterium (*Pseudomonas fluorescens*). They were all grown from pure culture onto discs of Whatmans No. 1 filter-paper (2.2 cm diam.) that had been autoclaved in sealed glass tubes containing a little distilled water. The discs were subsequently transferred to the surface of malt extract agar in Petri dishes, 8 discs per dish. The agar of 10 dishes was left untreated to obtain control discs, 10 were inoculated with 1 ml of a concentrated *Penicillium* spore suspension, 10 with 2 ml of a suspension of *P. fluorescens* in 2 % nutrient broth (containing 5 mg 1^{-1} Nystatin to eliminate fungal contamination), and 10 with small pieces of agar taken from an actively-growing culture of *M. hiemalis*. The plates were incubated at 25° C for 5 to 10 days until the microbes had developed over the paper discs. Fifty discs of each treatment were then removed and cleared of any adhering agar. Then 5 discs of each treatment plus 5 control discs were placed randomly in each of the 10 earthworm containers and their relative positions noted. After 24 h any discs missing were presumed to have been selected by the earthworms and dragged into their burrows.

Tests were made to establish that the earthworms were behaving normally under the experimental conditions imposed, and to investigate how they reacted to filter-paper discs. In the first of these the earthworms were supplied with 2.2 cm diam. discs of oak and ash leaves as well as sterile filter-paper. The ash and oak discs were taken from leaves that had weathered for 16 weeks in the field. It has been well established that ash leaves are preferred by earthworms to those of oak (EDWARDS and LOFTY 1972), so that the expected food preferences were ash > oak > filter-paper. The discs were soaked in distilled water to soften them and 5 of each type were placed in each container for 24 h. In the second test 20 paper discs were soaked in distilled water and 20 in Nystatin solution before being presented to the worms.

A guide to the amount of C present was obtained by ashing the experimental material at 400° C for 8 h on the assumption that half the total weight loss approximately estimates the proportion of C. The N content was determined more accurately by the micro-Kjeldahl method.

II. — RESULTS

Preliminary tests.

70 % of Nystatin discs and 80 % distilled water discs were taken in the experimental period, suggesting that Nystatin does not unduly impair the palatability of potential food to earthworms. The leaves and filter-paper discs were selected in their expected order of preference (ash 56 %; oak 14 %; filter-paper 4 %). These results indicate that the earthworms used in the experiments were behaving normally and exhibiting typical food preferences.

Microbial choice experiments.

The results of two experiments (Tab. I) indicate a preference for fungal over bacterial discs, and a preference for contaminated discs over the controls. A variance ratio test was applied to each set of experiments to determine the possible significance of the choices made, and the F values were calculated. In the first experiment both *Mucor* and *Penicillium* discs were taken by the earthworms at levels (99.9 %) significantly different from the control. In the second experiment only the number of *Mucor* discs differed significantly

TAB. I

Selection by *Lumbricus terrestris* of microbially contaminated cellulose (50 discs per treatment)

	Control	*Pseudomonas fluorescens*	*Penicillium* sp.	*Mucor hiemalis*
Experiment 1 % taken.........	2	14	34	16
Experiment 2 % taken.........	8	10	22	44

TAB. II

Estimates of C and N in experimental discs

Disc treatments	Disc d. wt. (mg)	C (%)	N (%)
Mucor..................................	50	51	0.35
Penicillium..............................	42	48	0.92
Pseudomonas.............................	39	53	Trace
Control..................................	34	50	not detected

(95 %) from the control. In neither experiment were the numbers of *Pseudomonas* discs taken significantly different from the control.

Chemical analyses.

Rapid analyses of C and N in contaminated and control discs were made to try to identify a correlation with the preferences observed. The fungal discs had a higher N content than either the bacterial or the control discs, whereas the percentage C present did not vary much according to treatment (Tab. II). The *Penicillium* discs contained more than twice as much N as the *Mucor* discs.

III. — DISCUSSION

Although the range of experiments performed was small, and the results are not conclusive, they do suggest that microbial contamination makes a potential food source attractive to worms and that the attraction of fungal contamination is greater than that of bacterial. The presence of a N resource may also enhance the palatability of food material.

WRIGHT (1972) showed that the presence of bacteria *(Pseudomonas aeruginosa)* on food material increased feeding rates of earthworms, especially when the bacteria were spread onto paper discs. A further indication of the association between earthworms, leaves and microbes was also given by WRIGHT who demonstrated that, of 46 partly-eaten leaves pulled from earthworm burrows, 37 showed growth of microbes. Of 54 uneaten leaves only 12 showed microbial growth. The lodging of leaves in the moist environment of the burrow probably enhances microbial growth. This development might make the leaf more attractive to the worms in one of two ways: the microbial tissue or cells might provide a useful food, or the microbes might condition the leaf in some way to make it more palatable to the worms.

SATCHELL (1958), PARLE (1963) and PIEARCE (1978) suggested that soil microbes may be a food source for some earthworms. MULLER (1950) thought that *Allolobophora caliginosa* fed mainly on fungal mycelia since it possessed a chitinase. BOLTON and PHILLIPSON (1976) measured the assimilation efficiency of the earthworm *Allolobophora rosea* and found it to be very low (consistently below 2.5 %) suggesting it was digesting only the microbial component of its food intake. However, NIELSEN (1962) concluded that earthworms do not possess a trehalase and since trehalose is an important storage carbohydrate of many fungi this may indicate that worms do not digest all the fungal components of their diet.

SATCHELL and LOWE (1966) suggested that microbial degradation of 'distasteful substances' is important in increasing the palatability of litter to earthworms during weathering, but were unable to explain the difference in acceptability of weathered litters in which these substances were not present. WRIGHT (1972) showed that leaves with an artificially doubled phenolic content did not reduce earthworm feeding rate in comparison with untreated leaves. Thus, SATCHELL's (1967) observation of an inverse correlation between the palatability of litter and its total polyhydric phenol content might better be expressed as a positive correlation between palatability and microbial development on the leaf. This viewpoint explains the differences in the acceptability of weathered litters containing no tannins, and also accounts for the preferences we observed.

If earthworms do not select contaminated cellulose as a microbial food source, and if the attractiveness of contamination is not brought about by microbial breakdown of distasteful substances, then why do earthworms find microbially-modified substrates so palatable ? A possible answer is that the microbes alter the physical nature of the leaves, making them more acceptable to the alimentary system of the worm, although SATCHELL and LOWE (1966) showed that there was little correspondence between the mechanical nature of leaf litter and its palatability. However, they also demonstrated a broad relationship between palatability, carbohydrate content, and N content and suggested that some taste substances, other than tannins, may affect the attractiveness of leaf litters to earthworms. It seems reasonable to suggest tentatively that microbial contamination of dead plant material releases phagostimulants attractive to earthworms and that, furthermore, this contamination makes available carbohydrate and N resources which the earthworms can more readily utilise.

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SUMMARY

Two soil fungi (*Mucor hiemalis* and *Penicillium* sp.) and a soil bacterium (*Pseudomonas fluorescens*) were cultured on filter-paper discs. Together with untreated control discs, they were presented to laboratory populations of *Lumbricus terrestris* which were allowed to make a selection. The earthworms found the fungus-contaminated discs to be the most palatable. It is postulated that fungal growth on food substrates may enhance the availability of those carboydrates and nitrogenous compounds which earthworms utilise.

RÉSUMÉ

Deux champignons (*Mucor hiemalis* et *Penicillium* sp.) et une bactérie (*Pseudomonas fluorescens*) du sol ont été cultivés sur des disques de papier filtre. Ces disques, de même que des témoins non traités, ont été présentés en laboratoire à des populations de *Lumbricus terrestris* qui ont porté leur choix principalement sur les disques supportant des champignons. Il est vraisemblable que la croissance des champignons sur le substrat nutritif augmente la disponibilité en composés carbohydratés et azotés dont se nourrissent les vers de terre.

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